Compatibility of *Trichoderma harzianum* with commercial herbicides

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**ABSTRACT**

Biological disease control has become popular in recent years and has established itself as a viable alternative to chemical control. However, the efficiency of biological control can be limited by the application technology. Thus, the objective of this work was to evaluate the compatibility of *Trichoderma harzianum* with three commercial herbicides. The fungus was incubated in culture medium supplemented with doses of three commercial herbicides and incubated for 4 days. The CI50 (concentration capable of inhibiting 50% of the mycelial growth of the fungus) was determined for each herbicide and compared with the recommended doses for field application. All herbicides tested reduced mycelial growth, with CI50 lower than the recommended doses. The data suggest low compatibility between *T. harzianum* and the tested herbicides.

**Keywords:** Atrazine, Biological Products, Potassium Glyphosate, Trifluralin.
RESUMO
O controle biológico de doenças se popularizou nos últimos anos e se estabeleceu como uma alternativa viável ao controle químico. No entanto, a eficiência do controle biológico pode ser limitada pela tecnologia de aplicação. Assim, o objetivo desse trabalho foi avaliar a compatibilidade de *Trichoderma harzianum* com três herbicidas comerciais. O fungo foi incubado em meio de cultura suplementado com doses de três herbicidas comerciais e incubado durante 4 dias. A CI50 (concentração capaz de inibir 50% do crescimento micelial do fungo) foi determinada para cada herbicida e comparada com as doses recomendadas para aplicação a campo. Todos os herbicidas testados reduziram crescimento micelial, com CI50 menores do que as doses recomendadas. Os dados sugerem baixa compatibilidade entre *T. harzianum* e os herbicidas testados.

Palavras-chave: Atrazina, Controle Biológico, Glifosato Potássico, Trifluralina.

RESUMEN
El control biológico de las enfermedades se ha popularizado en los últimos años y se ha establecido como una alternativa viable al control químico. Sin embargo, la eficacia del control biológico puede verse limitada por la tecnología de aplicación. Así, el objetivo de este trabajo fue evaluar la compatibilidad de *Trichoderma harzianum* con tres herbicidas comerciales. El hongo se incubó en medio de cultivo suplementado con dosis de tres herbicidas comerciales y se incubó durante 4 días. Se determinó la CI50 (concentración capaz de inhibir el 50% del crecimiento micelial del hongo) para cada herbicida y se comparó con las dosis recomendadas para aplicación en campo. Todos los herbicidas evaluados redujeron el crecimiento micelial, con una CI50 menor a las dosis recomendadas. Los datos sugieren baja compatibilidad entre *T. harzianum* y los herbicidas evaluados.

Palabras clave: Atrazina, Control Biológico, Glifosato Potásico, Trifluralina.

1 INTRODUCTION

Biological control agents are living organisms and are sensitive to the factors surrounding the application. It is important that techniques involving the use of biological products in the field ensure maximum biocontrol effect (Spadaro and Gullino 2005; Preininger et al. 2018; Pérez-García et. al 2011). The application technology for biofungicides must preserve the biological activity and mechanisms of action of the microorganism, allowing the biological agent to establish itself in the area and be able to compete with the pathogen (Cortés-Rojas et al., 2021).
Different microorganisms require specific application conditions (Bamisile et al., 2018). Mixing with pesticides depends on the compatibility between the biocontrol agent and the product to be used and the specific application conditions for both. Fungicides, insecticides, and herbicides can interfere with the action of microorganisms and reduce biocontrol efficiency (Gonçalves et al. 2018; Marcellin et al. 2018; de Araujo et al. 2021; Ramírez-Olier et al. 2019).

Application tank mixing is a common practice among farmers (Barroso et al., 2022; Gazziero, 2015), who use it as a way to reduce application operating costs. This may be a viable alternative to reduce costs and even increase the efficiency of biological control. However, decision making must be guided by thorough knowledge regarding the microorganisms involved, the application and management conditions, and the mixture to be made (Funahashi and Parke 2016; Jones et al. 2018; Gonçalves et al. 2018; Marcellin et al. 2018).

Pesticides may or may not interfere with biocontrol efficiency (Silva et al., 2018), so it is important to check the compatibility between the biological control agent and the chemical used. In order to understand the factors of application technology of biological control agents, the objective of this work was to evaluate the compatibility of *Trichoderma harzianum* with three herbicides.

### 2 MATERIAL AND METHODS

Three in vitro experiments were conducted to evaluate the effect of three herbicides on the mycelial growth of *Trichoderma harzianum*. The herbicides used were the commercial products listed in Table 1.

<table>
<thead>
<tr>
<th>Commercial Product</th>
<th>Active Ingredient</th>
<th>Concentration (g.L−1)</th>
<th>Manufacturer</th>
<th>Chemical Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primóleo®</td>
<td>Atrazine</td>
<td>400</td>
<td>Syngenta</td>
<td>G</td>
</tr>
<tr>
<td>Trifluralina Nortox Gold®</td>
<td>Trifluralin</td>
<td>450</td>
<td>Nortox</td>
<td>K1</td>
</tr>
<tr>
<td>Zapp Qi® 620</td>
<td>Potassium glyphosate</td>
<td>620</td>
<td>Syngenta</td>
<td>C1</td>
</tr>
</tbody>
</table>

Table 1. Herbicides used in the experiments. Source: The author, 2024
2.1 ISOLATION, GROWTH AND STORAGE CONDITIONS

The *Trichoderma harzianum* isolate came from the commercial product Trichodermil (CEPA ESALQ 1306 - MAPA 2007 Registration). The commercial formulation containing spores (2,109 conidia per mL) of the antagonist was deposited in Petri dishes containing BDA culture medium and incubated for seven days at 25 ± 2 °C temperature and 12-hour light photoperiod (Carvalho et al., 2014). After the incubation period, discs of mycelium 0.7 cm in diameter were removed and re-incubated to maintain pure cultures. All antagonism tests were performed with the 7-day-old *T. harzianum* colonies.

2.2 SENSITIVITY OF TRICHODERMA HARZIANUM TO HERBICIDES

To evaluate the effect of herbicides on the growth of *T. harzianum*, BDA culture media were prepared (Potato 200 g L⁻¹ + Dextrose 15 g L⁻¹ + Agar 15 g L⁻¹ + streptomycin sulfate 300 µg mL⁻¹) supplemented with 6 doses (10,000, 1,000, 100, 10, 1, and 0 mg L⁻¹) of active ingredient for each of the herbicides tested. Then, mycelium discs of 0.7 cm in diameter were placed in the center of Petri dishes (90 mm) containing culture medium, one disc per dish, and incubated at a temperature of 25 ± 2 °C and a 12-hour light photoperiod. At the end of the 4-day period, the colonies were measured on two orthogonal axes. The diameters were used to calculate the area of the colonies, considering the ellipse area equation (Eq. 1).

\[
\text{Colony area} = \frac{\pi \times \text{Diameter 1} \times \text{Diameter 2}}{4}
\]  

The colony area data were subjected to analysis of variance and regression analysis. Then the CI₅₀ was determined, that is, the concentration capable of inhibiting 50% of the mycelial growth of *T. harzianum*. The CI₅₀ was estimated by linear interpolation (Pazzagli et al., 2009), after determining the linear regression between mycelial growth area and herbicide dose (transformed to Ln).
To evaluate the possibility of combined application of the biological control agent with the tested herbicides, regression plots were plotted and included a representation of the lowest dose and highest volume of syrup recommended by the manufacturer in the respective package inserts. In this way, the sensitivity of the fungus to the doses of herbicides applied in the field can be visualized.

All in vitro trials were conducted in an entirely randomized design with five replicates. Each herbicide was considered a stand-alone experiment and evaluated separately. Statistical tests were performed with the help of R and Microsoft Office Excel Data Analysis add-in.

3 RESULTS

The doses of the herbicide atrazine had a significant effect (P<0.0001) on the growth of *T. harzianum* colonies (Figure 1). The calculated CI₅₀ for this mixture is 69.38 mg.L⁻¹, which is well below the lowest dose recommended by the manufacturer, which is 8,000 mg.L⁻¹.

Figure 1. Mycelial growth of *Trichoderma harzianum* submitted to doses of the herbicide atrazine.

P<0.0001; R²=63%.

\[ y = -3.823 \ln(x) + 48,165 \]

Source: The author, 2024
The herbicide trifluralin reduced the mycelial growth of *T. harzianum* (P<0.0001). The observed CI$_{50}$ for this interaction is 7.69 mg.L$^{-1}$ (Figure 2). The inhibition observed at such a low dose suggests a strong fungitoxic effect on the biocontrol agent. The lowest dose recommended by the manufacturer is 1,350 mg.L$^{-1}$, a point at which mycelial growth is about 3%.

Figure 2. Mycelial growth of *Trichoderma harzianum* submitted to doses of the herbicide trifluralin. P<0.0001; R$^2$=80%

In evaluating the effect of potassium glyphosate on *T. harzianum*, it was observed that the herbicide reduced the mycelial growth of the fungus (P>0.001). The CI$_{50}$ determined for this interaction is 9.64 mg.L$^{-1}$ (Figure 3). The lowest dose recommended by the manufacturer is 1,736 mg.L$^{-1}$, a point at which mycelial growth is about 22%.
4 DISCUSSION

The CI_{50} observed under atrazine doses was far from the recommended dose for field application. Atrazine can reduce the development of fungi that lack the mechanisms to metabolize its molecules (Emurotu and Anyanwu 2016). Because it is one of the most widely used chlorinated herbicides in the world, it would be interesting if biological control agents were compatible with atrazine (Rostami et al., 2021). And, although some Trichoderma species have the ability to degrade atrazine in soil (Pelcastre et al., 2013), the data indicate that this T. harzianum isolate is not compatible with this active ingredient.

The mycelial growth caused by trifluralin suggests a fungitoxic effect. The pathways for trifluralin degradation in soil are poorly known, and although some bacteria and fungi have the ability to dealkalize the herbicide molecules (Coleman et al., 2020), studies shown that trifluralin reduces the development of other Trichoderma species and that it may also impair the mycorrhization process (Peixoto et al., 2010; Santoro et al., 2014; Santos; Silva; Trazzi, 2021).
Glyphosate effect on fungus is different for each case. While some species of the fungus have their growth and sporulation reduced by the herbicide (Ramírez-Olier et al., 2021), others can easily degrade glyphosate molecules to utilize phosphorus (Arfarita et al., 2013; Kunanbayev et al., 2019). Glyphosate shows low compatibility with *Trichoderma* species and isolates. Interactions between the fungus and the herbicide suggest that there is little feasibility for tank mixing (Ali; Ramadan, 2019).

When considering the mechanism of action of the herbicides evaluated (Marchi, 2008), it is observed that atrazine acts specifically on the biochemical process of photosynthesis. It is possible that its fungitoxic effect is more related to the increase in herbicide dose (Chan Cupul et al., 2014). On the other hand, the effect of trifluralin and potassium glyphosate may be associated with their mechanism of action. Trifluralin acts on microtubule formation, preventing cell division, while Glyphosate acts on the synthesis of aromatic ring amino acids (Marchi 2008; Reis et al. 2013). It is possible that the inhibition of these metabolic activities is related to the observed reduction in the mycelial growth of *T. harzianum*.

5 CONCLUSION

The biological control agent *T. harzianum*, under the conditions of the experiment, had no compatibility with the commercial herbicides atrazine, trifluralin, and potassium glyphosate. There is a need for field studies on the positioning of this biological product in relation to the herbicides tested to evaluate the effect of herbicides on the efficiency of biological control.

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